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TITLE: Genomic Basis of Prostate Cancer Health Disparity Among African-American Men

PRINCIPAL INVESTIGATOR: Harry Ostrer, M.D.

RECIPIENT: Albert Einstein College of Medicine of Yeshiva University, Bronx, NY 10461

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Harry Ostrer, M.D.
harry.ostrer@einstein.yu.edu

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14. ABSTRACT
The hypothesis for this study is that copy number alteration (amplification and deletion) in a limited repertoire of genes Is highly predictive of prostate cancer metastasis. This signature is present in primary prostate cancers at the time of diagnosis and is enriched in the primary prostate cancers of African-American men, thus accounting for the health disparity of prostate cancer metastasis among them. The biological effect of these copy number events is to convey an escape from anoikis, as well as the other features that occur with metastasis. The current study will confirm this signature in prostate cancers that have been shown to metastasize , compared to those that have not and to determine the prevalence of this high-risk signature in the prostate cancers of African-American men matched for stage compared to those of European-American men. This study will also demonstrate that the signature can be detected in prostate cancer biopsies and correlated between the biopsy and associated tumor specimens. This study will answer an important question about the apparent health disparity of prostate cancer metastasis as well as develop a clinically useful tool that could be used to select treatment for men diagnosed with prostate cancer.

15. SUBJECT TERMS
Prostate cancer, health disparity, metastasis, African-American men, genomics, copy number alteration

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INTRODUCTION

Compared to European-American (EA) men, African-American (AA) men have a 2-fold greater risk of dying from metastatic prostate cancer (1-2). For both groups, proper categorization of prostate cancer biopsies as high or low-risk for metastasis at the time of diagnosis would optimize treatment, improving outcomes and minimizing toxicity. The Ostrer laboratory has demonstrated that the specific genes within metastatic prostate cancers have been altered by amplification (increase in the copy number) or deletion (decrease in the copy number) (3). These genes appeared to have been selected by the advantages that they conveyed to tumors, such as escape from cell death ('anoikis'). These amplified or deleted metastasis genes are enriched 2.5-fold in the primary prostate cancers of AA men – a degree of enrichment that is similar to the enhanced likelihood of metastasis. The current study is designed to confirm these observations about gene patterns predictive of metastatic potential in new cohorts of men for whom outcome data are available. The current study will also provide DNA sequence of the exomes (expressed part of the genomes) in a subset of these tumors and a risk model that can be used for categorizing newly diagnosed prostate cancers as high or low-risk for metastasis. These methods will be applied to prostate cancer biopsy specimens to demonstrate that they could be used at the time of diagnosis for prediction of outcome. This study will be beneficial to all men with prostate cancer, because it will provide a diagnostic tool that could be used for selection of therapy. It is especially beneficial for African-American men who have a greater likelihood of disease and metastasis and could provide a precise answer for the challenging problem of this health disparity.

KEYWORDS

Prostate cancer, metastasis, African-American men, health disparity, genomics, copy number alteration, predictive signature.

OVERALL PROJECT SUMMARY

Year 1: The main efforts during the first year of the project were review of the clinical data for the subjects in the study to verify their inclusion, selection of formalin-fixed paraffin-embedded (FFPE) tissue blocks, and macrodissection of tumor or normal tissue for genetic analysis. Notably, IRB approval was secured from Duke and authorized by the U.S. Army Medical Research and Materiel Command Human Research Protection Office. Cases were identified among men who received radical prostatectomy for prostate cancer and who had accurate long-term follow-up information. These that had distant metastases have been frequency matched to men cured by radical prostatectomy by age (within 5-years), race (EA vs. AA men), pathological stage (exact match), margin status (exact match), grade (Gleason score, exact match), surgery year (within 3 years), PSA (<10, 10-20, and >20) and location (North Carolina versus New York). There is currently no accepted definition of "cured" after surgery, since late recurrences occur and the cure by surgery subgroup will undoubtedly be confounded by high-risk primary tumors. For this study, we have used PSA <0.2 ng/ml five years after surgery as a surrogate marker for "cured" because: (a) PSA recurrences (PSA >0.2 ng/ml) are uncommon after 5 years and (b) Even when PSA recurrences do occur after 5 years, they are rarely fatal (4).

Among the identified cases, the Pathology Departments at the Durham Veterans Administration Medical Center (VAMC) and Montefiore Medical Center reviewed the pre-existing H&E slides for evidence of cancer. The pathologists selected the two blocks with the highest tumor content and one that was tumor free. We retrieved the corresponding FFPE tissue blocks and cut 12 slices each of 5 micron thickness. These sections were placed in 2 ml Eppendorf tube, bar-coded with a unique de-identified code for each patient and assembled for genomic analysis.

The biomarkers are copy number alterations (CNAs) detected by molecular inversion probe (MiPS) technology using the Affymetrix Oncoscan v2 SNP array developed specifically for genomic DNA samples extracted from FFPE tissues. This array has been applied to more than 5000 samples with an average pass rate of 92%. Among the features of the method are a wide dynamic range (0-60 copies) and interrogation of the entire genome by analysis of more than 335,000 markers. To assess the validity of our metastasis signature and MPS prediction model, we tested a Durham VAMC cohort that was made up of a group of primary tumors that metastasized following radical prostatectomy (mPT, n=14), a group of high-risk tumors that did not develop distant metastases (iPTLRs, n=6), and, a group of low-risk tumors that did not develop distant metastases (iPTHRs, n=7). The high-risk designation of the iPTHR groups was assigned based on whether the patient experienced biochemical recurrence and received adjuvant radiation and/or hormone therapy after surgery whereas the iPTLRs represent tumors of men that were considered low-risk and did not receive adjuvant therapy.

The MPS score was calculated for 27 of the 30 VAMC cohort samples that passed quality control filters (Figure 1, panel C) and shown to distribute as expected for mPTs versus iPTLRs showing significant differences as per Mann-Whitney test (p =0.01).

Thus, every step in the subject identification, tumor assessment, block retrieval, dissection, DNA extraction, Oncoscan v2 array and data analysis met our expectation and suggested that we could meet the goals of Specific Aim 1 of our study.

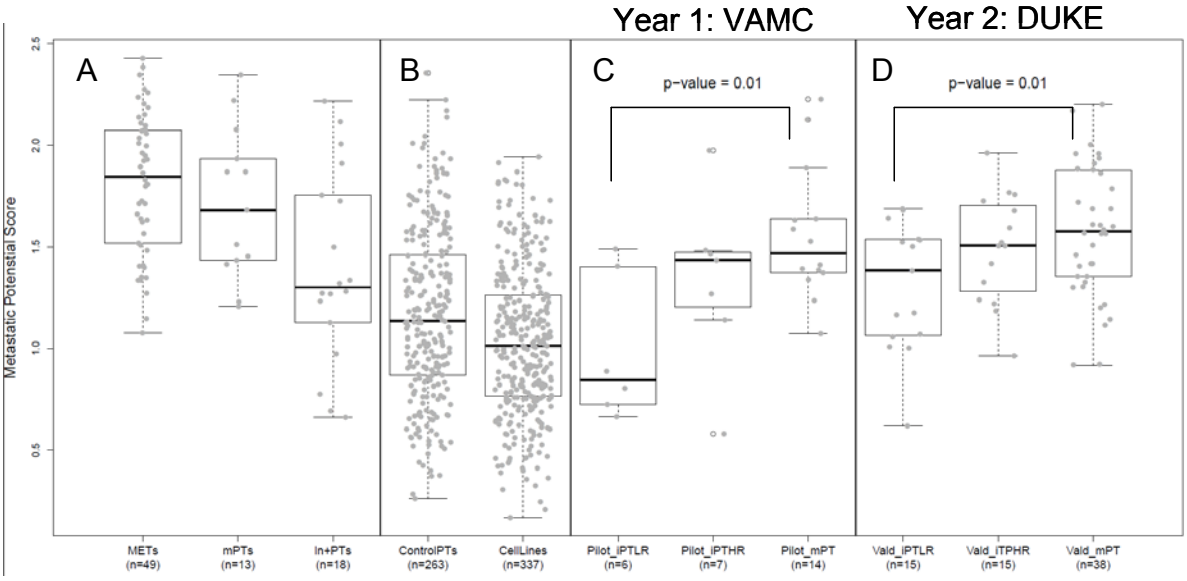

Figure 1. Boxplots of MPS score (Y-axis) of primary tumor samples from the Durham VAMC cohort pilot study (panel C) ***performed in year one*** and Duke University Medical Center cohort (panel D) ***performed in year two*** shown relative to previously studied cohorts (panels A/B) (3). METS are metastases. mPTs are primary tumors that went on to metastasize, iPTLR/iPTHR are low risk and high risk indolent tumors that have not metastasized after five years. Ln+PTs are tumors that spread to regional lymph nodes. Control PTs are primary tumors whose natural history is unknown. Cell lines are derived from tumors of various origins.

Year 2: We developed a new v3 array along with Affymetrix. The characteristics of this array were improved coverage around cancer genes and, specifically, our metastasis signature genes. An especially useful feature of this array is the utilization of a universal reference profile developed by Affymetrix that standardizes each experiment without the need for non-cancer "normal" samples run in the same batch. We validated this array by running 18 samples on v2 and v3 arrays and observed general concordance between the two platform versions and increased number of probes spanning gene regions and improved signal to noise at amplified or deleted parts of the genome on the v3 platform. Therefore, we were satisfied with the performance of the v3 array to transition our study assays to this platform.

We applied the v3 array to 80 samples 40mPTs (20 AAs/20 EAs) and 40iPTs (20 AAs/20 EAs) from an additional independent cohort from Duke University Medical Center (Figure 1, panel D). In preliminary analysis, of the 68 samples that passed quality control filters, we observed a significant increase in the MPS of mPTs over iPTLRs ($p = 0.01$).

Other advancements in year 2 include development of an automated H&E slide analysis procedure from scanned images which improves our ability to quantify tumor nuclei and area. When completed this procedure will assist in determining which samples are expected to yield enough tumor signal to call CNAs and calculate an MPS. Another statistical procedure (permute-MPS) was developed for purposes of determining whether sufficient signal is used to generate an MPS from each CNA profile. Samples not reaching significance in this test are considered non-informative because of technical noise, elevated tumor heterogeneity (with normal cells) or biology.

KEY RESEARCH ACCOMPLISHMENTS

Year 1: In the first replication study (n=30), preliminary analysis shows MPS can discriminate between mPTs and iPTLRs ($p = 0.01$) using Oncoscan V2 arrays.

Year 2: In a second replication study (n=80), preliminary analysis shows MPS can discriminate between mPTs and iPTLRs ($p=0.01$)

CONCLUSION

The salient feature of this study involves translation of the basic research of tumor biology into a risk model that can provide informed clinical decisions for men with prostate cancer and their physicians. This will determine whether prostate cancers are treated aggressively, because they are deemed to have high metastatic potential, or whether they are treated with active surveillance, because they are deemed indolent. These findings are now being applied to studying the health disparity of prostate cancer metastasis.

PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS

Publication

None for current performance period

Presentation

None for current performance period

INVENTIONS, PATENTS AND LICENSES

Patent application

None for current performance period

REPORTABLE OUTCOMES

Development of an accurate predictive score for risk of metastasis in prostate cancer surgical specimens.

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